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The many faces of filamin: A versatile molecular scaffold for cell motility and signalling

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The many faces of filamin: A versatile molecular scaffold for cell motility and signalling

Yuanyi Feng and Christopher A. Walsh

Filamins were discovered as the first family of non-muscle actin-binding protein. They are lage cytoplasmic proteins that cross-link cortical actin into a dynamic three-dimensional structure. Filamins have also been reported to interact with a large number of cellular proteins of great functional diversity, suggesting that they are unusually versatile signalling scaffolds. More recently, genetic mutations in filamin A and B have been reported to cause a wide range of human diseases, suggesting that different diseases highlight distinct filamin interactions.

Filamin was discovered as the first non-muscle actin binding protein almost 30 years ago. The three filamin isoforms in mammals (FLNA, B and C) are large cytoplasmic proteins that play important parts in cross-linking cortical actin filaments into a dynamic three-dimensional structure. In addition to filamentous actin, filamins have been reported to interact directly with more than 30 cellular proteins of great functional diversity. More recently, null and specific missense mutations in filamin A and B have been reported to cause a wide range of developmental malformations of brain, bone, limbs and other organs in humans, suggesting perhaps that different diseases highlight distinct filamin interactions. These diverse interactions show that filamin proteins are unusually versatile signalling scaffolds.

Mammalian filamin genes encode a family of high-molecular-weight, dimeric proteins that were first purified almost 30 years ago based on their ability to bind to the filamentous actin from non-muscle cells[1,2]. The filamin polypeptide chain, which has a relative molecular mass of 280,000, consists of an amino-terminal actin-binding domain (ABD) and a long rod-like domain of 24 repeated, anti-parallel β-sheets, interrupted by two roughly 30-amino-acid, flexible loops that are proposed to form hinge structures[3]. The dimerization of filamins though the last carboxyl-terminal repeat allows the formation of a V-shaped flexible structure that is essential for function[3,4].

Composed of two tandem calponin homology domains (CHD1 and CHD2), the ABD of filamins resembles many other actin binding proteins, such as β-spectrin, α-actinin, calponin and dystrophin, and it allows filamins to bind to filamentous actin and induce potent actin filament gelation[5–7]. Filamin can induce high-angle orthogonal branching and efficiently gather actin filaments into a three-dimensional gel *in vitro* by cross-linking actin filaments[1,3,8,9]. As three-dimensional orthogonal networks of actin filaments represent a characteristic cortical actin structure at the leading edge of migrating cells, filamins are believed to be essential for mammalian cell locomotion through stabilizing loose microfilament nets[10].

Outside of the ABD, the rod domain repeats of the filamin molecule potentially form sandwiches of β-sheets resembling the immunoglobulin domain, and they apparently can also function as interfaces for protein–protein interactions. Filamins have so far been reported to interact with over 30 proteins of great functional diversity (Fig. 1)[10]. Because many filamin-interacting proteins are membrane receptors for cell signalling molecules, filamins may also function as an important signalling scaffold by connecting and coordinating a large variety of cellular processes to the dynamic regulation of actin cytoskeleton.

Filamin (FLN) in mammals comprises a family of three members: filamin A, filamin B and filamin C. The genomic organization of the three filamin genes (*FLNA*, *FLNB* and *FLNC*) is highly conserved, and all three members are widely expressed during development, although *FLNC* shows more restricted expression in skeletal and cardiac muscles during adult life[11,12]. The three filamin proteins (FLNa, FLNb and FLNc) show 60–80% homology over their entire sequence with the exception of the two hinge regions, which show greater divergence[3,13,14]. Recent genetic evidence suggests that filamins are essential for human development, and mutations in both *FLNA* and *FLNB* have been associated with human genetic diseases with abnormal development of brain, bone, the cardiovascular system and many other organs. Although different filamin isoforms seem to have distinct roles in development, they may also share a great deal of functional similarity and confer genetic redundancies that lead to a wide degree of variance in the genetic syndromes associated by their mutations. This review describes diseases caused by mutations in both *FLNA* and *FLNB* and discusses the potential mechanisms of FLNa that are underlined by these genetic mutations in humans.

Yuanyi Feng and Christopher A. Walsh are in the Department of Neurology, Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, New Research Building Rm 266, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
e-mail: cwalsh@bidmc.harvard.edu

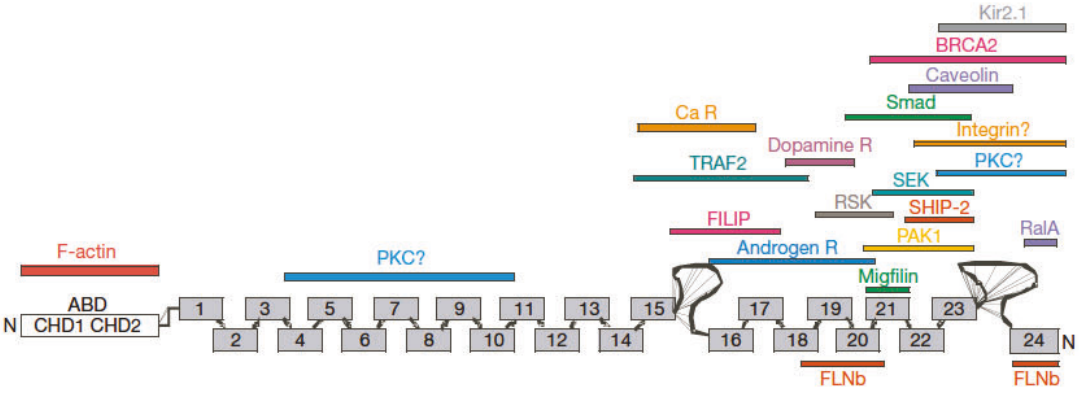

Figure 1 Schematic representation of filamin's molecular interactions. The ABD at the N terminus of filamin contains two calponin homology domains depicted as CHD1 and CHD2. The 24 repeats that follow the ABD most probably fold into antiparallel β-sheets and function as interfaces for protein–protein interactions. These repeats not only mediate the dimerization of filamin through the C terminus repeat 24, but also allow filamin to interact with over 30 proteins with great functional diversity. The figure presents a collection of proteins that have been described to interact with filamin directly. The approximate segments involved in each interaction on the filamin protein are depicted as well. In addition to what is presented in the figure, a number of proteins have also been found to bind filamin, although interaction domains on filamin have not been defined. These additional filamin interactors include the glycoprotein Ib–IX complex, the insulin receptor, γ- and δ-sarcoglycans, tissue factor, presenilin 1, Trio and Tc-mip.

Perventricular heterotopia

Although no genetic information on filamin function was available up until a few years ago, there has been a remarkable recent flow of information about diseases caused by filamin mutations. The first disease linked to filamin mutations in humans was an X-chromosome-linked brain malformation known as periventricular heterotopia[15,16]. A typical periventricular heterotopia brain shows the abnormal appearance of collections of neurons along walls of the lateral ventricle, where these neurons are originally generated during fetal development (Fig. 2). It seems that these ectopically localized neurons belong to the cerebral cortex, but failed to migrate to the correct cortical site during a brain developmental process called neuronal migration. Although the cerebral cortex of individuals with periventricular heterotopia is evidently missing many neurons that belong there, the intelligence of affected individuals is often normal or only mildly compromised. The major clinical syndrome of periventricular heterotopia is late-onset epilepsy that often starts in the second decade of life. Moreover, periventricular heterotopia is mostly found in females that have few male offspring and excessive miscarriages, suggesting X-linked dominant inheritance with prenatal lethality in hemizygous males.

Although originally identified as a central nervous system (CNS) malformation, individuals with periventricular heterotopia show a number of non-CNS phenotypes that are increasingly recognized. These include gut dysmotility and congenital cardiovascular abnormalities including persistence of ductus arteriosus and minor cardiac malformations, aortic aneurysms and premature strokes[17,18], whereas rare males with filamin mutations can have severe, lethal vascular defects and intractable haemorrhage[17,19,20]. Moreover, recent data suggest an association of periventricular heterotopia and *FLNA* mutation with an Ehler's-Danlos syndrome, characterized by connective tissue fragility, joint hypermobility and development of aortic dilation in early adulthood[21] (Sheen *et al.*, see *note added in proof*).

Periventricular heterotopia results from FLNA loss-of-function mutations

FLNA mutations in individuals with periventricular heterotopia usually result in abnormal mRNA splicing or early truncation of the FLNa protein[18,19,22] (Fig. 3). Although some missense mutations are also seen, periventricular heterotopia is generally believed to reflect loss of function of one *FLNA* allele. On the basis of the role of FLNa in cortical actin stabilization and remodelling, a straightforward model of *FLNA* function in the developing cerebral cortex can be formed: it is required for the dynamic regulation of filamentous actin at the leading edge of migrating neurons, with a lack of FLNa immobilizing neurons completely and thus preventing them from leaving the ventricular zone. Likewise, the fact that FLNa can interact with membrane receptors that are required for cell adhesion suggests an alternative but related model in which FLNa-deficient neurons may fail to attach to the radial glia cells that normally guide their migration and are therefore unable to migrate out of the ventricular zone. According to both neuronal migration models, the presence of cortical neurons with two different behaviours (migration or failure to migrate) may relate to differential X-inactivation of the mutant or normal X chromosome in the normal or heterotopic neurons, respectively, because cells in the female brain randomly inactivate one or the other X chromosome.

Although the migration-based models are supported by an essential role of FLNa in cell motility and membrane stability (see below), certain genetic and clinical observations associated with periventricular heterotopia are not explained by these models. For example, if FLNa-deficient neurons are indeed completely arrested in the cortical ventricular germinal zone, then the cerebral cortex of periventricular heterotopia females should only receive about 50% of the neurons that are generated in a normal brain, assuming that the X-inactivation is random. But in most periventricular heterotopia cases the volume of the heterotopic neurons is small relative to the normally located neurons. A further puzzle in understanding how *FLNA* mutations cause periventricular heterotopia comes from a recent genetic analysis that has identified *FLNA* mutations in several male affected individuals[17,19,20]. Although mutations in these male individuals with periventricular heterotopia may cause partial loss of function of *FLNA*, some of the affected individuals do not seem to be somatic mosaics for the *FLNA* mutations, but nonetheless can survive into adulthood. These male individuals with periventricular heterotopia have a pattern of nodules very similar to females, implying that *FLNA*-mutant neurons either never migrate or migrate normally, but never stop halfway. Whereas heterodimerization of FLNa and FLNb through repeat 19–20 may in part compensate the loss of *FLNA* function during cerebral cortical development in the male individuals with periventricular heterotopia[12], it is still unclear why hypomorphic mutations or genetic

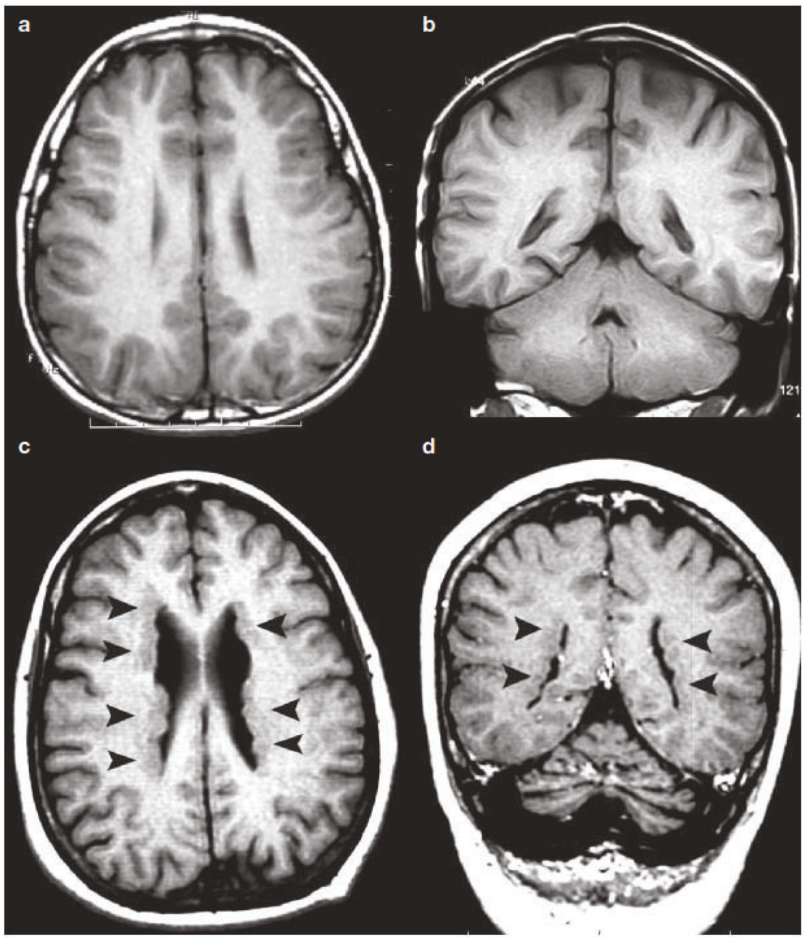

Figure 2 Periventricular heterotopia. (**a**–**d**) Magnetic resonance imaging (MRI) images of a normal human brain (**a**, **b**) and of a patient with periventricular heterotopia (PH) (**c**, **d**). Images are taken in the axial plane (**a**, **c**) essentially parallel to the brim of a hat, or in the coronal plane (**b**, **d**). In contrast to the smooth ventricular surface shown in the normal brain, a rough zone of cortical neurons with the same signal intensity as normal cortex (indicated by arrowheads) is seen along the lateral walls of the lateral ventricles, representing neurons that have not migrated to the cortex during early brain development.

compensation might produce an 'all or none' phenotype in which some *FLNA*-mutated cells migrate all the way to the cerebral cortex while the others are completely immobile. More interestingly, a recent report of a male infant born to a periventricular heterotopia mother, who died one week after birth due to multiple organ defects, showed that, in spite of widespread periventricular nodules, large areas of the cerebral cortex were normally structured[20]. That the affected individual's mother carried an 8-base deletion that leads to the truncation of the FLNa at the rod domain repeat 14, suggests that at least some neurons with severe non-sense mutations of *FLNA* are still able to migrate to the cerebral cortex. Therefore, additional mechanisms independent of simple cell motility may ultimately determine the pathogenesis of *FLNA*-mutation-induced periventricular heterotopia.

Apart from an essential role in regulating cortical actin structures, FLNa has also been implicated in regulating many cellular signalling pathways. Therefore, other models that reflect FLNA as a cell signalling scaffold may also be promising in understanding the development of periventricular heterotopia. Among these are the possibilities that periventricular heterotopia results from failure of programmed cell death of collections of neuroblasts within the periventricular germinal matrix. Alternatively, continued proliferation of neural precursor cells beyond the programmed window of neurogenesis (when the radial glia scaffold can guide neuronal migration) could produce excess neurons that lack the normal guidance for migration. One *FLNA* mutation associated with periventricular heterotopia in a male truncates the FLNa protein at amino-acid 2305. The truncated FLNa protein lacks the C-terminal dimerization domain and several repeats required for the interaction of FLNa with membrane receptors such as integrins and cell signalling molecules including protein kinase C (PKC), the mitogen-activated protein (MAP) kinase SEK, Smad and the small GTPase RalA[23–27]. Perhaps the molecular mechanism that causes the development of periventricular heterotopia is specifically dependent on one or more of these molecular interactions. However, no direct evidence on the actin-independent role of FLNA in the pathogenesis of periventricular heterotopia is currently available. Further elucidation of the molecular pathways underlined by *FLNA* in cerebral cortex development will rely on experimental genetic models, such as an FlnA-knockout mouse, which has so far not been reported.

Otopalatodigital (OPD) spectrum disorders

The spectrum of phenotypes caused by *FLNA* mutations has recently broadened considerably with the recognition that four other X-linked human disorders, including otopalatodigital syndrome types 1 (OPD1) and 2 (OPD2), frontometaphyseal dysplasia (FMD) and Melnik-Needles syndrome (MNS), are all caused by localized mutations in the *FLNA* gene[28]. Typically, OPD1 is manifest with conduction deafness, cleft palate, characteristic facial malformations and a generalized bone dysplasia, including wide and broad thumbs and big toes, long metacarpals, severe scoliosis and congenital dislocation of the hips and knees[29]. The OPD2

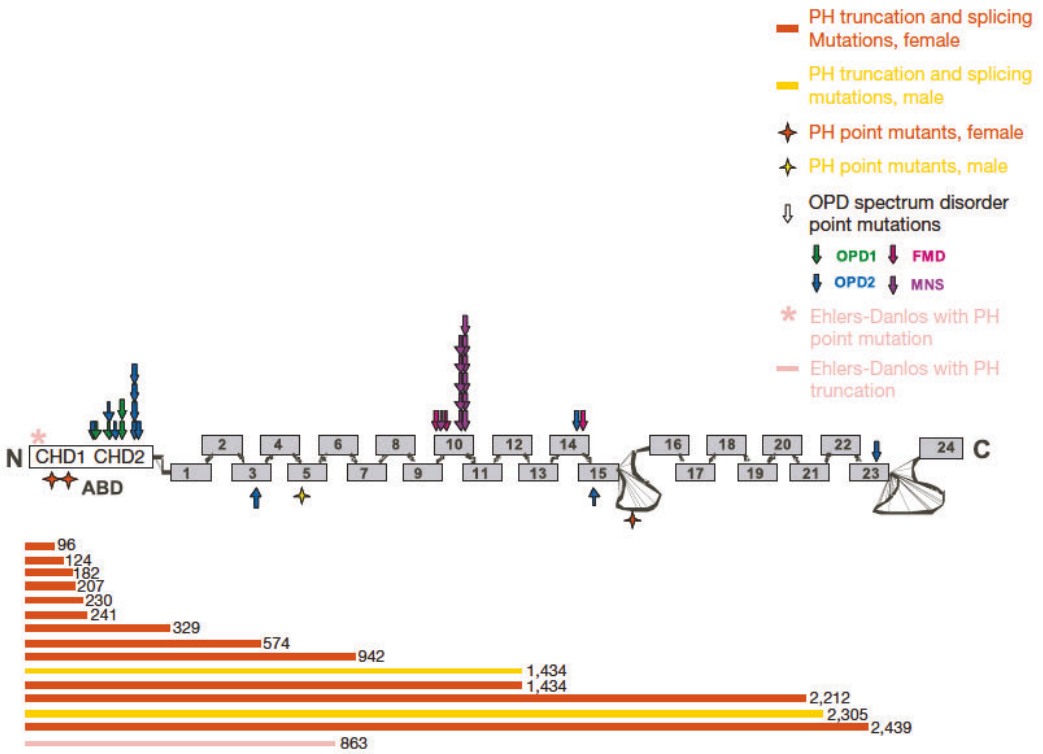

Figure 3 *FLNA* mutations identified in various human genetic disorders. A summary of mutations in *FLNA* and their correlation with various human diseases identified so far. Whereas a large number of mutations identified in periventricular heterotopia (PH) are nonsense and splicing mutations, all mutations identified in OPD spectrum disorders are missense mutations that cluster in remarkably small regions of the FLNa protein. The non-overlapping phenotype between periventricular heterotopia and OPD spectrum disorders and the segregation of genetic mutations associated with various diseases suggest that different mutations of *FLNA* may highlight distinct interactions as well as functions of filamin.

syndrome resembles OPD1 but is a more severe disorder, and is accompanied by microcephaly and mental retardation[30,31]. Whereas OPD1 is mostly seen in males, OPD2 occurs in both males and females[28].

The FMD disorder also represents a morphogenetic defect of bone, characterized by striking overgrowth of frontal facial bones. Similar to OPD, some individuals with FMD also show deafness, digital anomalies and osteodysplasia[32]. More males are affected by FMD and females are usually only mildly affected. Likewise, MNS also overlaps with OPD2 in showing malformed skull and craniofacial structures, but also shows irregular constrictions in the ribs, deformed clavicles, scapula and pelvis, and curved long bones. MNS mostly affects females, with the frequent observation of skewed X-inactivation[33,34]. The sons of affected women usually die shortly after birth with a wide range of defects in the kidneys, urethra and atrioventricular canal, and complete malrotation of the gut[35].

OPD spectrum disorders reflect specific missense mutations of FLNA

In contrast to loss-of-function or partial loss-of-function mutations that cause periventricular heterotopia, *FLNA* mutations in OPD, MNS, or FMD are invariably missense point mutations that cluster in remarkably small regions of the FLNa protein. The occurrence of OPD1 reflects missense mutations in the second CH domain of the actin binding region of the protein; OPD2 mutations occur either here, or in repeats 3, 14 or 15. A point mutation of E245K (in CHD2) occurs in many OPD2 families, and mutation of P207L presents in two families with OPD1. All known FMD mutations affect the N-terminal portion of repeat 10 or repeat 14. All known MNS mutations affect the C terminus of repeat 10 (Fig. 3). Point mutation A1188T was found in five families and S1199L was found in six families of MNS.

Although there is certainly overlap between OPD, MNS and FMD, with generalized bone dysplasia involving craniofacial structures, digits and long bones common to all, the degree to which mutations causing the three syndromes are segregated is striking. Even more interesting is the fact that none of these three disorders are associated with periventricular heterotopia, or any other definable neuronal migration disturbance of the brain. So far, only a single case has been suggested to share periventricular heterotopia as well as elements of OPD spectrum disorders, evidently by a point mutation that simultaneously causes a point mutation of a highly conserved leucine (L2439M) and a splicing defect that produces a shortened *FLNA* transcript[36].

These remarkable patterns of mutation beg the question of what, if anything, these distinct mutant alleles are trying to tell us about the role of FLNa in brain and bone development. The distinguishable phenotypes and specific genotype–phenotype correlations produced by different *FLNA* mutations suggest that periventricular heterotopia represents a loss-of-function phenotype, whereas OPD-spectrum disorders result from gain-of-function changes in FLNa. The mutations in the CHD2 domain are predicted to alter the interface involved in actin binding, and similarly distributed mutations in the CHD2 of α-actinin actually enhanced actin binding[37]; therefore, OPD mutations in the CHD2 domain may increase the actin binding of filamin and either disorganize actin or create toxic products that function in a dominant-negative fashion. However, repeats 10, 14 and 15, which contain many of the missense mutations in OPD spectrum disorders, are among the least characterized segments of the FLNa protein in terms of their function and binding partners. Further identification of proteins that directly interact with these regions may provide insight into the mechanism of *FLNA* function in OPD and in associated bone development processes.

Mutations in FLNB disrupt bone morphogenesis

Recently, mutations in *FLNB* have been found to cause a class of diseases with abnormal vertebral segmentation, joint formation and skeletogenesis[38]. Spondylocarpotarsal syndrome (SCT) is an autosomal-recessive disorder characterized by short-trunk dwarfism of postnatal onset, unsegmented thoracic vertebrae, and carpal bone fusions. Larsen syndrome is an autosomal-dominant and genetically heterogenous disorder characterized by multiple joint dislocations, craniofacial abnormalities and accessory carpal bones. Atelosteogenesis I and III (AOI and AOIII) are autosomal dominant lethal skeletal dysplasia with vertebral abnormalities, disharmonious skeletal muscles and poorly modelled long bones and joint dislocations. All of these syndromes are caused by various mutations in the *FLNB* gene. Heterozygous mutations that cause Larson syndrome, AOI and AOIII have so far only been found in the actin-binding domain (ABD) of *FLNB*. These mutations may function in dominant fashion by enhancing actin-binding analogons to the OPD mutations in *FLNA*. In contrast, point mutations in the rod-domain repeats 5, 6 14, 15, 20 and 22 have been identified to cause SCT as either homozygous or compound heterozygous mutations, and may reflect simple loss of function[38].

It is interesting to note that the phenotype of these *FLNB*-mutation-caused disorders have some overlap with the OPD spectrum diseases caused by *FLNA* mutations. Because *FLNB* and *FLNA* are both widely expressed and the two proteins are highly similar in both structure and molecular interactions, the potential genetic and functional redundancy and compensation of different filamin isoforms thus adds another layer of complexity to our understanding of the diseases caused by filamin mutations.

Conclusion

Our understanding of filamins has advanced rather notably through studying the molecular interactions and human genetic diseases associated with their mutations. However, the phenotypic complexity showed by *FLN* mutations in various human diseases suggests the possibility that filamin has more complicated roles in mammalian development than just simply being required for cell locomotion. Thus, further investigation of the role of filamin in the modulation of both cell signalling and cell mechanics during organogenesis in multiple tissues will be a new challenge. After 30 years of discovery and intensive study, our understanding of the molecular mechanism of filamin in the context of human development is still just beginning.

Note added in proof: While this review was in the press, Sheen et al. *was accepted for publication (*Neurology, *in the press).*

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